

# Histamine Intolerance-Like Symptoms in Healthy Volunteers after Oral Provocation with Liquid Histamine

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## ABSTRACT

*Histamine in food at non-toxic doses has been proposed to be a major cause of food intolerance causing symptoms like diarrhea, hypotension, headache, pruritus and flush ("histamine intolerance"). Histamine-rich foods such as cheese, sausages, sauerkraut, tuna, tomatoes, and alcoholic beverages may contain histamine up to 500 mg/kg. We conducted a randomized, double-blind, placebo-controlled cross-over study in 10 healthy females (age range 22–36 years, mean  $29.1 \pm 5.4$ ) who were hospitalized and challenged on two consecutive days with placebo (peppermint tea) or 75 mg of pure histamine (equaling 124 mg histamine dihydrochloride, dissolved in peppermint tea). Objective parameters (heart rate, blood pressure, skin temperature, peak flow) as well as a total clinical symptom score using a standardized protocol were recorded at baseline, 10, 20, 40, 80 minutes, and 24 hours. The subjects received a histamine-*

*free diet also low in allergen 24 hours before hospitalization and over the whole observation period. Blood samples were drawn at baseline, 10, 20, 40, and 80 minutes, and histamine and the histamine-degrading enzyme diamine oxidase (DAO) were determined. After histamine challenge, 5 of 10 subjects showed no reaction. One individual experienced tachycardia, mild hypotension after 20 minutes, sneezing, itching of the nose, and rhinorrhea after 60 minutes. Four subjects experienced delayed symptoms like diarrhea (4×), flatulence (3×), headache (3×), pruritus (2×) and ocular symptoms (1×) starting 3 to 24 hours after provocation. No subject reacted to placebo. No changes were observed in histamine and DAO levels within the first 80 minutes in non-reactors as well as reactors. There was no difference in challenge with histamine versus challenge with placebo. We conclude that 75 mg of pure liquid oral histamine—a dose found in normal meals—can provoke immediate as well as delayed symptoms in 50% of healthy females without a history of food intolerance. (Allergy and Asthma Proc 25:305–311, 2004)*

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**A**ll fermented food is prone to be rich in biogenic amines as a result of microbial activity in food decaying processes.<sup>1</sup> Hence, histamine, cadaverine, and putrescine are used as surrogate markers for the grade of food spoilage.<sup>2</sup> In contrast to the other aforementioned amines, endogenously produced histamine also plays an important role in many physiologic and pathologic processes such as in allergy, gastroenterology, and neurotransmission.<sup>3</sup> Histamine has been shown to be the causative agent in mackerel, tuna, and marlin toxic fish poisoning.<sup>4</sup> However, some patients

report intolerance reactions to food at non-toxic doses of histamine.<sup>5</sup>

The term "histamine intolerance" was introduced to describe symptoms like diarrhea, hypotension, headache, pruritus, and flush following the ingestion of foods rich in histamine, such as cheese, sausages, sauerkraut, tuna, and tomatoes.<sup>6</sup> These foods usually contain doses of histamine and other biogenic amines less than 100–225 mg/kg, a dose reported as "pretoxic" via oral intake.<sup>7</sup> Therefore, these reactions should be classified as non-toxic, non-immune-mediated, pharmacological food intolerance<sup>8</sup> according to the recommendations of the 1995 position paper of the European Academy of Allergy and Clinical Immunology.<sup>9</sup>

In humans, 68 to 80% of orally administered histamine is absorbed in the gut.<sup>10</sup> The two enzymes, diamine oxidase (DAO) and histamine-*N*-methyltransferase (HNMT), catabolize histamine as well as other biogenic amines in the human intestine.<sup>11</sup> DAO is localized mainly in the epithelial tissues of the intestine and the kidney.<sup>12</sup> The intestinal DAO protects the organism from histamine absorbed from exogenous/nutritional and endogenous sources (mainly subepithelial mast cells).<sup>13</sup> Histamine intolerance has been thought to be caused by a decreased DAO activity.<sup>14,15</sup>

Histamine also has been reported to be an important agent in intolerance to alcoholic beverages, such as red wine.<sup>16,17</sup> However, the role of histamine in histamine intolerance has never been clearly defined because patients also report symptoms in response to alcoholic beverages low in histamine, such as white wines and some sorts of champagne. Other pathogenic ingredients like tannins,<sup>18</sup> sulfite<sup>19,20</sup> and ethanol itself<sup>21,22</sup> have been proposed as possible causes.

However, some authors have suggested that histamine is the causative agent in intolerance to foods as well as to alcoholic beverages. Wantke *et al.*<sup>23</sup> observed a transient increase in serum DAO activity upon challenge with red wine substituted with 20 mg histamine dihydrochloride. Nevertheless, no clinical symptoms appeared in the healthy volunteers. Kanny *et al.*<sup>24</sup> reported a direct gastro-duodenal histamine challenge with 120 mg histamine dihydrochloride via nasogastric tube. Five of the seven patients suffering from chronic urticaria showed symptoms like diarrhea, urticaria, headache, accelerated heart rate, and drop in blood pressure. Sjaastad and Sjaastad do not report any clinical symptoms in their 1974 and 1977 histamine-metabolism studies in humans with a 200 and a 100 mg oral histamine diphosphate load, respectively (equaling 68 and 34 mg pure histamine base).<sup>10,25</sup>

We conducted a randomized, double-blind, cross-over, placebo-controlled histamine challenge with 75 mg of pure histamine base (equaling 124 mg histamine dihydrochloride) in healthy volunteers to investigate whether orally administered histamine alone can cause symptoms of histamine intolerance. Five of ten subjects had symptoms associated with histamine intolerance after the histamine challenge and none after the challenge with placebo. However, in these healthy individuals we observed no alterations in

the plasma histamine levels or the DAO serum activity upon histamine challenge.

## PATIENTS AND METHODS

This study was conducted in a randomized, double-blind, placebo-controlled, cross-over manner. Ten healthy, non-pregnant females (age range 22–36 years, mean  $29.1 \pm 5.4$ ) were hospitalized at the Department of Dermatology at the Krankenanstalt Rudolfstiftung, Vienna. The probands received a histamine-free diet low in allergen 24 hours before hospitalization and over the whole observation period. They were orally challenged in the morning before receiving breakfast on two consecutive days, with placebo (peppermint tea) on the 1st day and 75 mg of pure histamine (equaling 124 mg histamine dihydrochloride, Reag Ph Eur Merck, Germany, order number 104370, dissolved in peppermint tea) on the 2nd day or vice versa—depending on randomization. Objective parameters (heart rate, blood pressure, skin temperature, peak flow) and a total clinical symptom score (Table I, modified after Bock *et al.*<sup>26</sup>) using a standardized protocol were recorded at baseline and at 10, 20, 40, 80 minutes, and 24 hours. Blood samples were drawn at baseline and at 10, 20, 40, and 80 minutes. Plasma histamine was assessed by radioimmunoassay (Immuno-tech, Marseille, France) and the diamine oxidase activity was determined by the <sup>14</sup>C putrescine method.<sup>27</sup>

The protocol was approved by the local ethics committee of the hospital Rudolfstiftung and the probands gave informed written consent to participate in the study.

## RESULTS

Half of the subjects (5 of 10) reacted to the histamine challenge with a total symptom score (Table I) of more than the cutoff level 3 (Fig. 1), whereas none reacted to placebo. Four of five probands experienced delayed symptoms appearing between 3 and 24 hours after the ingestion of histamine (Table II). Only subject no. 9 experienced immediate sneezing, nasal itch, and headache after 1 hour. This subject also showed an increase in pulse rate by 12 (85/minute to 97/minute) and a drop in systolic and diastolic heart pressure by 9 mmHg after 20 minutes. Individual no. 8 also suffered from delayed symptoms from histamine but the overall score reached only 3, and therefore the provocation was judged negative.

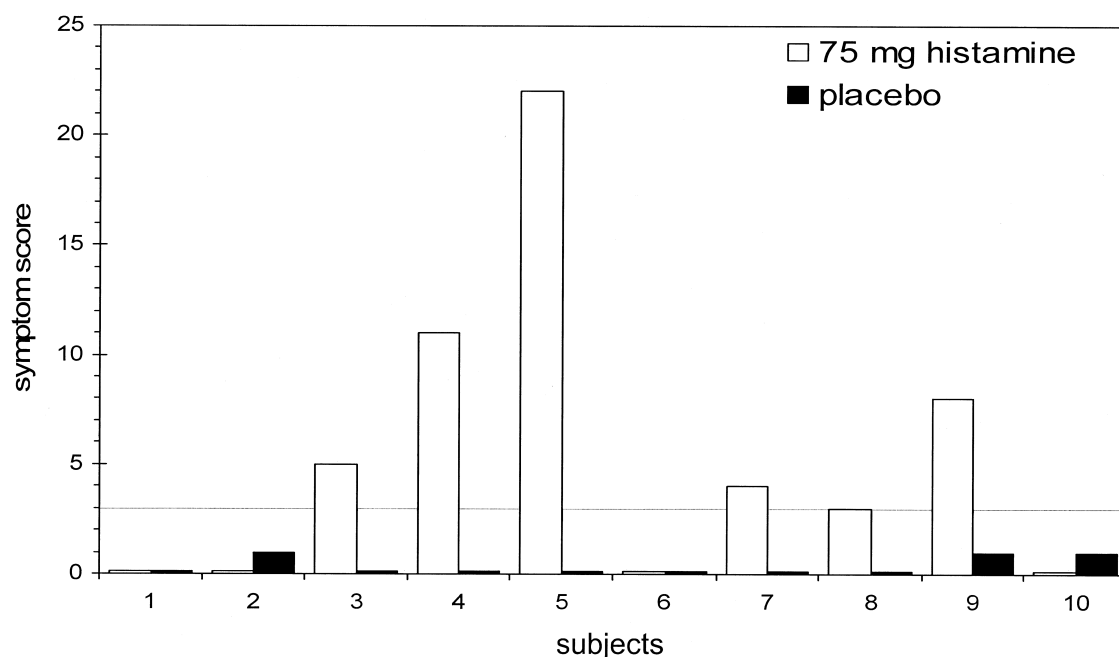
With the exception of subject no. 9, all probands with a positive provocation test reported gastro-intestinal symptoms (4 of 5), like diarrhea (4×), flatulence (3×), and loose stools (1×). Headache (3×), pruritus (2×), and ocular symptoms (1×) were the other symptoms observed upon histamine challenge. Interestingly, we never observed urticaria and pulmonary reactions.

There was no significant difference in any of the objective parameters (heart rate, blood pressure, skin temperature, peak flow) between histamine and placebo challenge on the one hand, and reactors and non-reactors on the other

TABLE I

Symptom Score (Modified After Bock 1988). Total Scores &gt;3 Were Counted As Positive

|   |         |   |    |
|---|---------|---|----|
| Severe systemic reactions   |         | diarrhea (multiplied with number of stools) |    |
| anaphylaxis/anaphylactoid reactions   | max. 15 | loose                                       | ×1 |
| bronchial asthma (FEV1 < 75%) or laryngeal edema + urticaria  | 5 each  | watery                                      | ×2 |
| hypotension and tachycardia (fall in blood pressure >30 mmHg + heart rate >130/min or increase in heart rate >20/min) | 5 each  | bloody                                      | ×3 |
| Organ-specific symptoms   |         | general distress                            |    |
| bronchial   |         | mild  | 1  |
| subjective  |         | moderate                                    | 2  |
| nasal congestion  |         | severe                                      | 3  |
| unilateral  | 1       | very severe                                 | 4  |
| bilateral   | 2       | gingival edema                              | 2  |
| mouth breathing   | 3       | vomit number                                | ×2 |
| objective   |         | flatulence                                  | 2  |
| rhinorrhea  |         | cardio-vascular                             |    |
| occas. sniffing   | 1       | only objective                              |    |
| requires tissues 3–5×/h   | 2       | hypotension/fall in blood pressure          |    |
| requires tissues >5×/h  | 3       | >20 mm Hg                                   | 6  |
| sneezing  |         | >30 mm Hg                                   | 10 |
| occasional  | 1       | tachycardia/increase in heart rate within   |    |
| 3–5×/h  | 2       | 1 hour                                      |    |
| >5×/h   | 3       | >10/min                                     | 2  |
| itch (nose, throat)   |         | >20/min                                     | 4  |
| occasional  | 1       | >30/min                                     | 8  |
| nose rubbing  | 2       | skin  |    |
| continuous  | 3       | subjective                                  |    |
| bronchial obstruction   |         | pruritus                                    |    |
| mild  | 1       | mild  | 1  |
| expiratory wheezing to auscultation   | 3       | scratching >2 min                           | 2  |
| FEV1 <75%   | 4       | contin. scratching, excoriations            | 3  |
| ocular  |         | objective                                   |    |
| subjective  |         | erythema                                    |    |
| itch/burning  |         | faint, few                                  | 1  |
| mild  | 1       | moderate, <50% of area                      | 2  |
| occasional eye rubbing  | 2       | generalized, >50%                           | 3  |
| continuous eye rubbing  | 3       | sensibility                                 |    |
| objective   |         | paresthesia                                 | 1  |
| conjunctivitis  |         | tickling                                    | 1  |
| erythema  | 1       | heat sensation                              | 1  |
| erythema + swelling   | 2       | urticaria/angioedema                        |    |
| bilateral swelling  | 3       | <3 hives                                    | 1  |
| gastro-intestinal   |         | 3–10 hives                                  | 2  |
| subjective  |         | generalized                                 | 3  |
| abdominal pain  |         | generalized rash                            | 3  |
| mild  | 1       | immune system                               |    |
| moderate  | 2       | body temperature                            |    |
| severe  | 3       | febrile 37.5–38°C                           | 1  |
| nausea  | 1       | fever 38–39°C                               | 2  |
| objective   |         | fever >39°C                                 | 3  |
|   |         | central nervous system                      |    |
|   |         | headache                                    |    |
|   |         | mild  | 1  |
|   |         | severe                                      | 2  |
|   |         | migraine                                    | 3  |



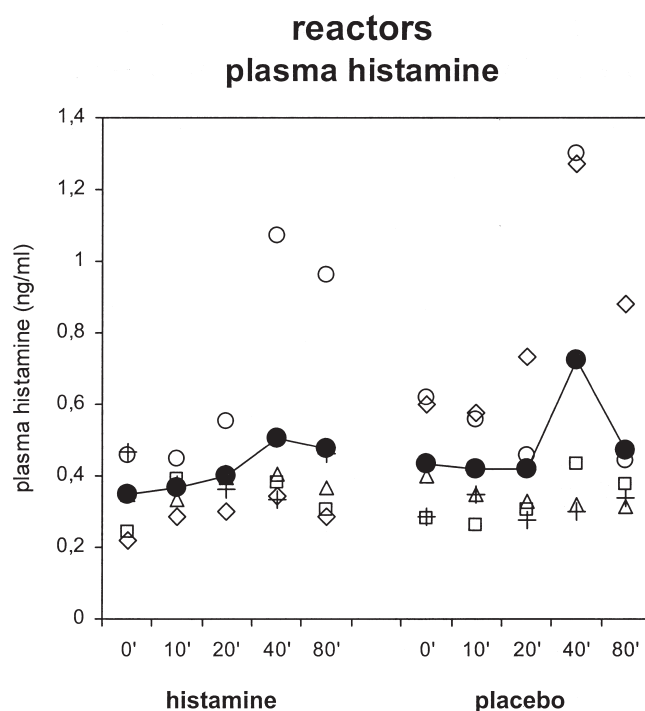
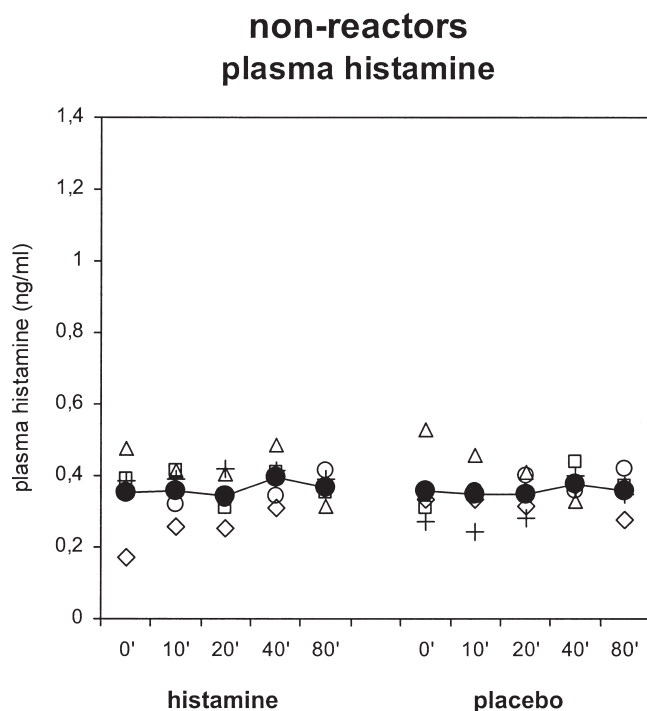
**Figure 1.** Total symptom score in 10 healthy female volunteers after oral provocation with 75 mg histamine and placebo, respectively. Five of 10 reacted positive to provocation with histamine, none to placebo. Total scores >3 were counted as positive.

**TABLE II**

**Symptoms According to the Symptom Score (Table I) after Oral Provocation with 75 mg Pure Histamine in 10 Healthy Volunteers**

|                           | Subject |   |          |         |         |   |         |         |         |    | Total |
|---------------------------|---------|---|----------|---------|---------|---|---------|---------|---------|----|-------|
|                           | 1       | 2 | 3        | 4       | 5       | 6 | 7       | 8       | 9       | 10 |       |
| Severe systemic reactions |         |   |          |         |         |   |         |         |         |    | 0/10  |
| Nasal                     |         |   |          |         |         |   |         |         |         |    |       |
| blocked nose              |         |   |          |         |         |   |         |         |         |    | 0/10  |
| rhinorrhea                |         |   |          |         |         |   |         | 6h      | 1h      |    | 2/10  |
| sneezing                  |         |   |          | 3h (1×) |         |   |         | 6h (1×) | 1h (5×) |    | 3/10  |
| itch                      |         |   |          | 3h      |         |   |         |         | 1h      |    | 2/10  |
| Cardio-vascular           |         |   |          |         |         |   |         |         | 20min   |    | 1/10  |
| Pulmonary                 |         |   |          |         |         |   |         |         |         |    | 0/10  |
| Ocular                    |         |   |          |         |         |   |         |         |         |    |       |
| itch                      |         |   |          |         | 12h     |   |         |         |         |    | 1/10  |
| erythema                  |         |   |          |         | 12h     |   |         |         |         |    | 1/10  |
| edema                     |         |   |          |         | 12h     |   |         |         |         |    | 1/10  |
| Gastro-intestinal         |         |   |          |         |         |   |         |         |         |    |       |
| nausea/vomiting           |         |   |          |         |         |   |         |         |         |    | 0/10  |
| diarrhea                  |         |   | 5h (1×)  | 3h (2×) | 7h (5×) |   | 3h (1×) |         |         |    | 4/10  |
| loose stools              |         |   | 24h (3×) |         |         |   |         |         |         |    | 1/10  |
| flatulence                |         |   |          | 5h      | 10h     |   | 5h      |         |         |    | 3/10  |
| Skin                      |         |   |          |         |         |   |         |         |         |    |       |
| pruritus                  |         |   |          | 5h      | 12h     |   |         |         |         |    | 2/10  |
| flush                     |         |   |          |         |         |   |         |         |         |    | 0/10  |
| urticaria                 |         |   |          |         |         |   |         |         |         |    | 0/10  |
| Headache                  |         |   |          |         | 11h     |   |         | 6h      | 1h      |    | 3/10  |
| Total score               | 0       | 0 | 5        | 11      | 22      | 0 | 4       | 3       | 8       | 0  |       |

Watery diarrhea beginning 3–7 hours after provocation was the most frequent symptom. Only one patient (no. 9) showed immediate reactions to the provocation and none of the subjects had severe systemic reactions. Following provocation with placebo, no patient had a positive total score of more than 3 points. Three patients (nos. 2, 9, 10) reached 1 point in total score.



**Figure 2.** Plasma histamine levels in non-reactors ( $n = 5$ ) and reactors ( $n = 5$ ) after oral provocation with 75 mg histamine and placebo, respectively (Closed circles: mean values).

hand (data not shown). The same was true for plasma histamine and serum DAO activity levels (Figs. 2 and 3). The elevated histamine values found in some serum samples probably are artifacts due to technical difficulties during blood collection, as they occurred after provocation with histamine as well as placebo.

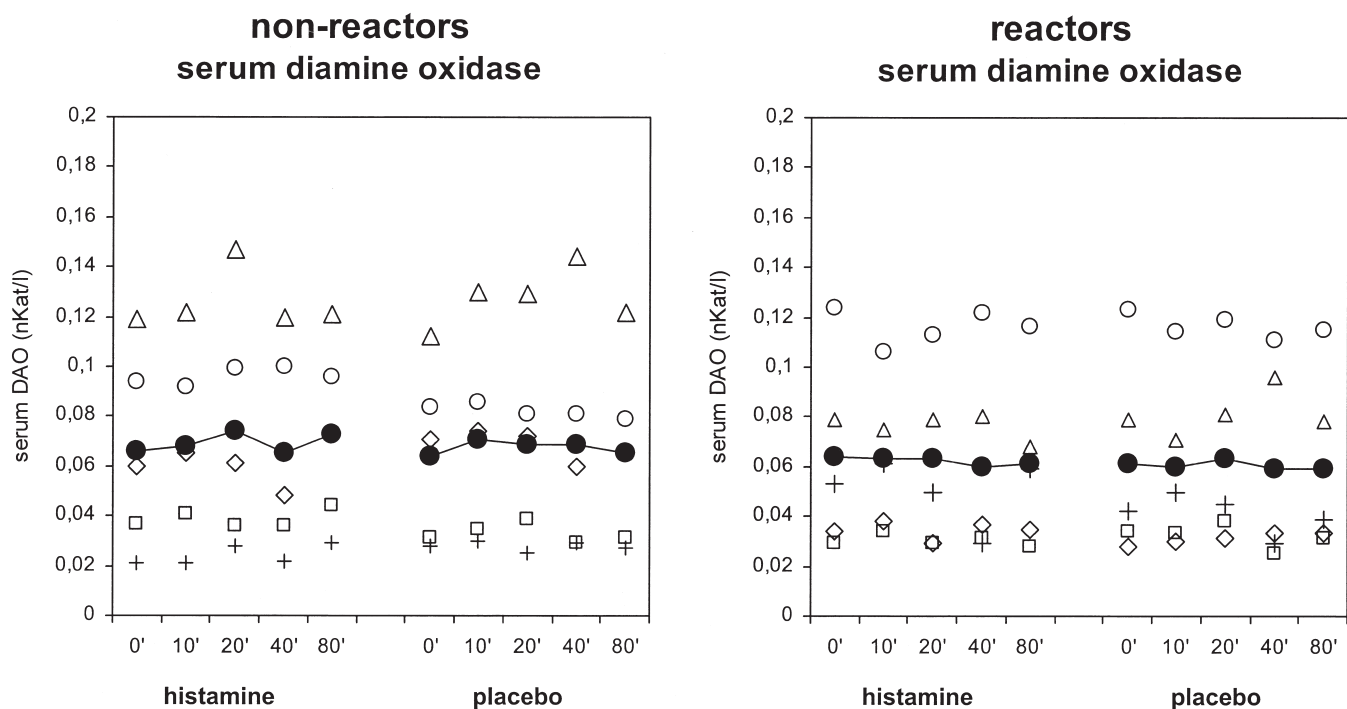
## DISCUSSION

In this study, a single oral dose of 75 mg of pure liquid histamine base (equalling 124 mg histamine dihydrochloride) could provoke immediate or delayed symptoms in 50% of healthy volunteers. Kanny *et al.* report that in 16 patients with a history of wine intolerance, a red wine with a high histamine content did not provoke symptoms more often than a wine sample low in histamine.<sup>28</sup> The histamine-rich red wine could not provoke any symptoms in a healthy control group.<sup>29</sup> They therefore suggest that histamine is not responsible for intolerance to wine. In the present study, the observed symptoms in healthy volunteers resemble those encountered in histamine intolerance. Hence, we conclude that histamine is a causative agent in histamine intolerance.

The diagnosis of histamine intolerance is based mainly on the patient's history,<sup>6</sup> especially on the patient's intolerance to histamine-rich foods.<sup>5</sup> However, the determination of histamine and DAO activity levels in the plasma has been suggested as being of additional value in the diagnosis of histamine intolerance<sup>6</sup> either as pathogenic or as surrogate markers. Theoretically, histamine intolerant patients might suffer from a variety of abnormalities, such as:

- High, transitional, systemic histamine concentrations leading to the aforementioned gastrointestinal and also to systemic symptoms like urticaria and hypotension. High plasma histamine levels could be reached, for example, through a decreased DAO activity on the inner mucosal surface of the intestine. Recently, certain polymorphisms in the DAO gene product have been shown to be associated with inflammatory bowel diseases like Crohn's disease and ulcerative colitis.<sup>30</sup> The decreased DAO activity might also come from a transitional pharmacological DAO inhibition<sup>31,32</sup> although such inhibition may be rather short-lived *in vivo*.<sup>33</sup> This decrease of histamine decaying activity could lead to a prolonged histamine "survival" in the gut. Consequently, histamine would reach lower gut regions in a much delayed manner, leading to the unexpected delayed reactions observed in patient no. 5. In this scenario of decreased histamine decaying activity, we should expect a decreased plasma DAO activity. Alternatively, a very rapid histamine uptake or an increased histamine permeability of the intestinal mucosa could circumvent the protective intestinal DAO activity:<sup>13</sup> plasma DAO levels should be normal. Histamine overloads, found in food intoxication,<sup>4</sup> would also lead to symptoms at normal DAO plasma levels. Finally, increased endogenous histamine production, as found in systemic mastocytosis or food allergy, would go along with either normal or elevated plasma DAO levels.
- A higher histamine sensitivity through an increased amount of histamine receptors or an increased histamine response of





**Figure 3.** Serum diamine oxidase activity in non-reactors ( $n = 5$ ) and reactors ( $n = 5$ ) after oral provocation with 75 mg histamine and placebo, respectively (Closed circles: mean values).

the histamine receptors at normal histamine levels (either through per se higher response or through up-regulation of histamine receptors): We would expect symptoms at normal plasma histamine and serum DAO levels.

- A direct histamine action on gastrointestinal histamine receptors without a necessary systemic histamine resorption, again at normal histamine and DAO levels. This might explain why we did not observe any alteration of the plasma histamine and DAO activity levels in the healthy subjects of this study. The following histamine receptors are abundant in the gut: the H1-receptor on smooth muscle and endothelial cells,<sup>34</sup> the H2-receptor on gastric parietal, smooth muscle, and endothelial cells,<sup>34</sup> small amounts of the mainly CNS-bound H3-receptor on peripheral nerves of the intestine,<sup>34,35</sup> and the H4 in the small intestine and mast cells.<sup>35</sup> Even systemic effects may be explained by direct gastro-intestinal histamine H1-receptor interaction. Radhakrishnan and Sharma<sup>36</sup> report a fall in blood pressure after gastro-intestinal histamine challenge in a rabbit model. The animals reacted with a significant fall in blood pressure without affecting heart rate and respiration. This effect was reversible by H1-blockage as well as by splachnicotomy, but not by vagotomy. The authors conclude that H1 action in the gut can lead to direct modulation of the sympathetic nervous system.

This study was performed on healthy volunteers and therefore we cannot predict a potential outcome in histamine-intolerant patients. However, in these healthy volun-

teers, 4 of the 5 reacting subjects suffered from delayed symptoms and only 1 from immediate symptoms. The organs most often affected were gut (4 of 10), central nervous system (3 of 10), and the nose (3 of 10). The predominant appearance of gastro-intestinal symptoms and the delayed appearance of the symptoms could be explained through a direct histamine/histamine-receptor interaction in the lower parts of the gut.

Ohtsu *et al.*<sup>37</sup> introduced a knock-out mouse model with animals that cannot synthesize histamine endogenously and that show a reduced number and consequently a reduced functionality in their mast cells. Within only 7 days of a histamine-rich diet, histamine tissue levels reached nearly 50% of the wild-type animals.<sup>38</sup> *In vitro*, histamine also restored the releasing capacity of the histamine-lacking bone marrow-derived mast cells to 40% in comparison to the wild type. These mice data show that orally administered histamine is stored in body tissues and can also augment the histamine-releasing capacity of mast cells. It may therefore be speculated that a histamine-free diet could deplete mast cells in human beings because part of the stored tissue histamine comes from exogenous sources.

Hence, these new molecular data may support the histamine-free diet concept in histamine-intolerant patients published by Wantke *et al.* nearly a decade ago.<sup>5</sup> They observed an improvement in food-intolerant and chronic headache patients after a 4-week diet avoiding high histamine-containing food. They hypothesized that the depletion in endogenous histamine stores could explain the curative effect.

It seems to be surprising that the rather low dose of 75 mg pure histamine base—a dose found in normal meals<sup>6</sup>—can provoke any symptoms in healthy subjects. In this study, histamine was administered in pure and liquid form in the morning before breakfast to rule out any interaction with other substances. Probably, this experimental setting is very artificial because even in cases of high nutritional histamine doses, histamine comes along with fatty acids, other amino acids, water, glucose, *etc.*, substances that all may modify the intestinal histamine uptake. However, threshold levels for histamine in natural food currently considered as non-toxic (100–225 mg/kg<sup>7</sup>) may simply be too high. Further provocation studies in histamine-intolerant patients should give more insight into the pathology of histamine intolerance.

## CONCLUSION

**S**eventy-five milligrams of pure liquid histamine—a dose found in normal meals—can provoke histamine intolerance-like symptoms in healthy subjects.

## ACKNOWLEDGMENT

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# Histamine intolerance: lack of reproducibility of single symptoms by oral provocation with histamine: A randomised, double-blind, placebo-controlled cross-over study

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**Histaminintoleranz: Fehlende Reproduzierbarkeit der einzelnen Symptome durch orale Provokation mit Histamin: Eine randomisierte, doppelblinde, Placebo-kontrollierte cross-over Studie**

**Zusammenfassung.** *Hintergrund:* Der Terminus Histamin-Intoleranz steht für eine ganze Reihe von Symptomen an unterschiedlichen Effektororganen, die nach dem Genuss Histamin-reicher Nahrung auftreten. Das Studienziel war, Histamin-assoziierte Beschwerden durch die Provokation mit einer standardisierten Menge an Histamin zu objektivieren und zu quantifizieren und zu prüfen, ob oral verabreichte Diaminoxidase (DAO) das Beschwerdeausmaß beeinflusst.

*Patienten und Methoden:* Vier Institutionen in Österreich nahmen teil. Es wurden Patienten rekrutiert, die den Verdacht auf Histamin-Intoleranz äußerten. Zunächst erfolgte eine offene orale Provokation mit 75 mg Histamin in Tee. Alle Probanden, die dabei Beschwerden entwickelten, wurden in den verblindeten Teil der Studie eingeschlossen und erhielten in drei weiteren verblindeten, einem cross-over Design folgenden Provokationsschritten Kombinationen aus Histamin-freiem und Histamin-hältigem Tee mit Diaminoxidase- und Placebo-Kapseln. Es wurden Haupt- und Nebensymptome (stärkste und weniger starke Beschwerden an den einzelnen Effektororganen auf einer 10-Punkte-Skala) definiert, die Reproduzierbarkeit dieser Beschwerden in Bezug auf die jeweiligen Effektororgane

geprüft, die Beschwerden weiters in ihrer Gesamtheit (Summe der Beschwerdepunkte aller Effektororgane) erfasst und Veränderungen nach Supplementation von DAO beurteilt.

*Ergebnisse:* 39 von 56 Patienten reagierten in der offenen Provokation und wurden in den verblindeten Studienabschnitt eingeschlossen. Haupt- und Nebensymptome waren dabei nicht zu reproduzieren, die Probanden reagierten quasi zufällig. Allerdings brachte die Einnahme von DAO eine statistisch signifikante Reduktion von Beschwerden (definiert durch den Rückgang der aufsummierten Gesamtpunkte) im Vergleich zur Placebo-Gruppe.

*Schlussfolgerung:* Die Ergebnisse erscheinen kontrovers. Möglicherweise reagieren Histamin-intolerante Personen zu unterschiedlichen Zeiten mit unterschiedlichen Symptomen. Die Reproduzierbarkeit definierter Symptome allein würde dann nicht genügen, um eine Histamin-Intoleranz zu bestätigen. Am Kollektiv zeigte sich weiters, dass die Einnahme von DAO-Kapseln im Vergleich mit Placebo-Kapseln zu einer statistisch signifikanten Abschwächung von Beschwerden nach Histamin-Provokation führte.

**Summary.** *Objectives:* The term histamine intolerance stands for a range of symptoms involving various effector organs after the consumption of histamine-rich food. Our intention was to objectify and quantify histamine-associated symptoms and to analyse whether oral administration of the histamine-degrading enzyme diamine oxidase (DAO) caused a reduction of symptoms.

*Patients and methods:* Four Austrian centres participated. Patients suspected to be histamine intolerant were

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recruited. The first step consisted in the open oral provocation of these patients with 75 mg of liquid histamine. Patients who developed symptoms were tested in a randomised double blind crossover provocation protocol using histamine-containing and histamine-free tea in combination with DAO capsules or placebo. Main and secondary symptoms (strongest and weaker symptoms based on a ten-point scale) were defined, the grand total of all symptoms of the individual provocation steps was determined and changes in symptoms after administration of DAO were measured.

**Results:** Thirty nine patients reacted to the open histamine provocation and were enrolled in the blinded part. Here, both the main and secondary symptoms were not reproducible. Subjects reacted sometimes unexpectedly and randomly. Regarding the total symptom scores, the differences between the three treatment groups were statistically significant. The intake of DAO demonstrated a statistically significant reduction of histamine-associated symptoms compared to placebo ( $P=0.014$ ).

**Conclusions:** Oral provocation with 75 mg of liquid histamine failed to reproduce histamine-associated single symptoms in many patients. One may suggest that histamine-intolerant subjects reacted with different organs on different occasions. As a consequence, reproducibility of single symptoms alone may not be appropriate to diagnose histamine-intolerance whereas a global symptom score could be more appropriate. The fact, that the intake of DAO capsules compared to placebo led to a statistically significant reduction of total symptom scores, may indirectly point in the same direction.

**Key words:** Biogenic amines, DAO, diamine oxidase, histamine intolerance, oral provocation.

## Introduction

Histamine, an alkaline biogenic amine with a molecular weight of 111 daltons, represents a highly reactive messenger substance (IUPAC - International Union of Pure and Applied Chemistry - name 1H-imidazole-4-ethylamine). This decarboxylation product of histidine is detectable in all body tissues, especially in mast cells and basophil granulocytes [1]. Histamine is stored in cytoplasmic granules and can be released quickly by degranulation. However, histamine is not only produced by the body but also constantly ingested with food to different degrees [2]. Hundreds of goods produced with bacteria regularly contain histamine. They include for example wine, cheese and Sauerkraut. In addition, histamine develops during the storage process of protein-containing food (canned fish and sausage, etc.) [3]. It is the responsible agent in fish poisoning [4]. Some patients describe food intolerance that occurs with subtoxic histamine doses. The term "histamine intolerance" was introduced as common denominator for symptoms such as abdominal pain, flatulence, diarrhoea, headache, pruritus, blepharidemas, urticaria, rhinorrhoea, dysmenorrhoea, respiratory obstruction, tachycardia, extrasystoles and hypotension occurring after the consumption of histamine-rich foods [5].

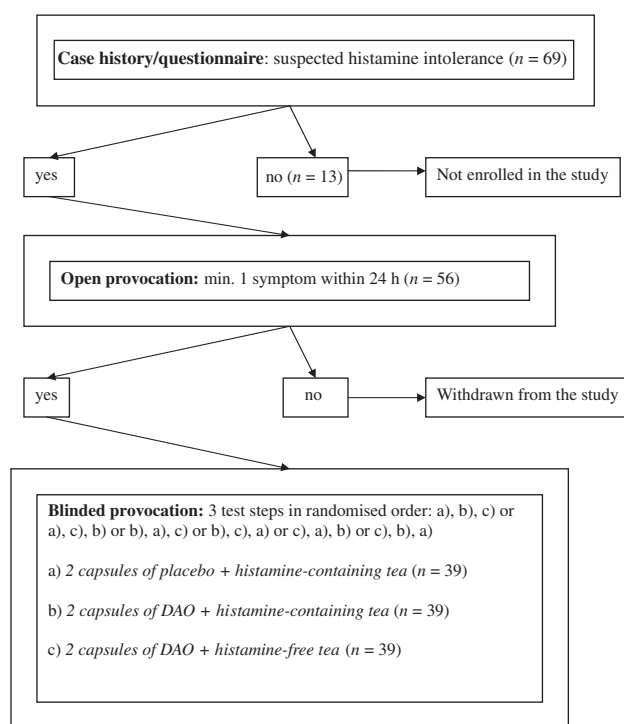
Histamine is degraded by two enzymes. Histamine-N-methyltransferase (HNMT, EC 2.1.8) is an enzyme predominantly located intracellular in blood cells and the liver [6]. Diamine oxidase (DAO, EC 1.4.3.6) is mainly produced by enterocytes and is used for the degradation of extracellular histamine [7]. Thus histamine ingested with food is largely degraded in the intestines [8]. When passing through the intestinal mucosa, additional histamine is degraded by the DAO located there. 6-hydroxydopa [9] and pyridoxal phosphate (vitamin B<sub>6</sub>) [10] serve as co-factors of DAO.

Histamine intolerance is attributed to an imbalance of histamine and DAO [11]. The DAO activity can be reduced by other biogenic amines, alcohol and its decomposition product acetaldehyde as well as various drugs [12].

Nevertheless, the symptoms of histamine intolerance are not clearly defined and not always identifiable. The purpose of the present study was to examine whether the oral provocation with a standardised histamine quantity in a selected patient sample yields reproducible subjective symptoms and whether the ingestion of commercially sold oral diamine oxidase (PelLind®) reduces or suppresses the symptoms.

## Patients and methods

Four Austrian allergological outpatient units of dermatological departments participated in a multicentre, double-blind, placebo-controlled crossover study: Graz, Innsbruck, Linz and Salzburg. The study was conducted in accordance with the principles of Good Clinical Practice (GCP) corresponding to the ICH guidelines (International Conference on Harmonisation of drugs and medical devices). A positive vote was obtained from an inde-



**Fig. 1.** Schematic illustration of study sequence including the number of participants of each step ( $n$ =number)

pendent ethics committee (protocol number 274/06). Before enrolment, every patient signed a declaration of consent after being provided with thorough verbal and written information.

Figure 1 contains a schematic illustration of the study sequence. In the first step patients who had repeatedly observed intolerance reactions after consuming histamine-rich food were selected. The enrolment age was between 18 and 75 years. Exclusion criteria were pregnancy, coronary heart disease, instable hypertension, bronchial asthma and regular H1 blocker intake. The patient history was recorded by means of a standardised questionnaire. Those patients who had regularly developed symptoms consistent with histamine intolerance (HIT) after the intake of food were invited to participate in the study.

The study sequence was divided into two phases: the first phase consisted of an open provocation with 75 mg of pure histamine in 100 mL of peppermint tea, to identify histamine-sensitive persons, and to objectify and to quantify the symptoms reported by the patients. In addition, this step was used to define the main symptom, i.e. the one of four symptoms patients marked as the strongest on a 10-point scale. Ten points were awarded for the maximum degree of symptoms. Less pronounced symptoms were classified as secondary symptoms. This 10 point symptom scale was used for the four symptoms I) headache, II) symptoms involving the skin (pruritus, erythema, and urticaria), III) symptoms involving the mucous membranes (itching, erythema/enanthema, and rhinorrhoea), and IV) gastrointestinal symptoms (nausea, vomiting, abdominal pain, flatulence, and diarrhoea). The symptoms were evaluated 90 min and 24 h after the oral provocation. Patients who did not develop any symptoms after the open provocation were excluded.

Patients who reached at least one symptom were subjected to a double-blind and randomised crossover provocation test. They were randomised according to a scheme defined before the study. Three provocation steps followed, consisting of a) 2 capsules of placebo + histamine-containing tea, b) 2 capsules of DAO + histamine-containing tea and c) 2 capsules of DAO + histamine-free tea assigned in random order. The DAO capsule manufacturer supplied one package each for every subject. Configurations a), b), and c) were randomly assigned to the colours green, blue and orange and the prepared packages provided to the test centres.

DAO (PelLind®) capsules are a gastric acid resistant nutrition supplement for patients with histamine intolerance. One capsule contains 0.25 mg of protein extract with natural DAO, which was extracted from pigs' kidneys, and holds an activity of 10,000 HDU (histamine degrading units) and an amount of 11 mg of vitamin C, and mainly microcrystalline cellulose and gelatine. In placebo capsules, the protein extract with DAO was replaced by microcrystalline cellulose. The provocations were administered after a 24-hour histamine-free diet at an interval of at least 48 hours and at most 10 days. The symptoms I) headache, II) symptoms involving the skin, III) symptoms involving the mucous membranes and IV) gastrointestinal symptoms were evaluated after 90 minutes and 24 h and were used to define the possible main symptom as well as secondary symptoms as it was done after the open prov-

ocation. The evaluation after 90 min was performed by the subject with the support of a test physician; the evaluation after 24 h by the subject alone. The evaluations after 90 min and 24 h were recorded separately for every symptom and later summarised to a single score for statistical calculation.

The primary objective was defined as the reduction of the main symptom score based on the 10-point-scale. An improvement by 40% or more was considered a good outcome and an improvement by 30–39% a satisfactory result. Other deviations of the score were referred to as not satisfactory. The secondary objective was defined as the reduction of the symptom score of the most severe secondary symptom (points awarded analogous to the main symptom score) and the tertiary objective was defined as reduction of the total score of all indicated symptoms.

The reproducibility of symptoms observed with open provocation was referred to as exploratory criterion. The symptom score of the blinded provocation 2 capsules of placebo + histamine-containing tea was used as comparison. A score deviation by plus/minus two points on the 10-point scale was deemed good reproducibility.

## Statistical methods

Ten points were awarded for the maximum degree of symptoms per target region (symptoms I–IV) and time (90 min and 24 h), and 2 points for the minimum degree of symptoms. One point was awarded for no symptoms.

The overall symptom score was calculated by adding the symptom points of 4 target organs at 2 points of time.

Consequently, the highest possible total score per person for each of the three test conditions and the open provocation was 80 points. Based on this scale, persons with a total score of 8 points are deemed “symptom-free”.

In the descriptive part, the specific values (case number, median, minimum and maximum) were calculated for each of the three test conditions (Table 1). In addition, the distribution was visualised with box plots (Fig. 2).

The recovery of symptoms was statistically tested with the calculation of a rank correlation (Spearman Rho) between the individual localisations of the open provocation and the ones of the condition placebo + histamine-containing tea.

Non-parametric methods are used for the inference statistical evaluation.

The regular distribution analysis was performed according to Kolmogorov–Smirnov and to Shapiro–Wilk. The rank test according to Friedman was used for the evaluation of the entire model. Multiple paired comparisons were performed according to Wilcoxon.

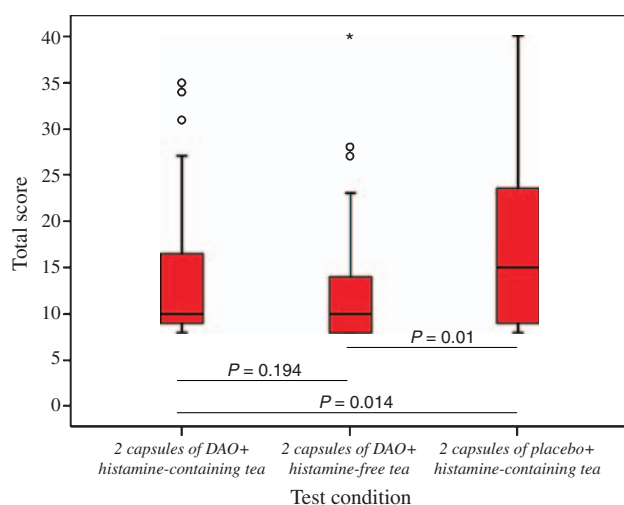
## Results

In total 56 subjects underwent the open provocation. 39 (35 females (average age 47 years), 4 males (average age 39 years)) out of 56 (69.6%) displayed at least one symptom

**Table 1.** Specific values of total scores for the 3 test conditions

|                                    | <i>n</i> | Median | 25th percentile | 75th percentile | Minimum | Maximum |
|------------------------------------|----------|--------|-----------------|-----------------|---------|---------|
| DAO + histamine-containing tea     | 39       | 10     | 9               | 17              | 8       | 35      |
| DAO + histamine-free tea           | 39       | 10     | 8               | 14              | 8       | 40      |
| Placebo + histamine-containing tea | 39       | 15     | 9               | 24              | 8       | 40      |

*n*: number of patients in the blinded part.



**Fig. 2.** Box plots for the 3 test conditions. The boxes represent the 1st and 3rd quartile and the median. Outliers and extreme outliers are marked by rings or stars, respectively. The difference is statistically significant (according to Friedman). *P*-values resulted from paired comparison of the 3 test conditions according to Wilcoxon (significance test)

and were enrolled in the blinded part of the study (subject distribution: Graz 13, Innsbruck 14, Linz 5 and Salzburg 7).

25 of these 39 (64.1%) subjects developed at least one symptom after a blinded provocation with *DAO+ histamine-free tea*.

Nine of 39 (23.1%) did not have a reaction to the combined administration of *placebo+ histamine-containing tea*. However, seven of this group (78%) responded with at least 1 symptom to the test configuration *DAO+ histamine-free tea*. Number and percentage of subjects with at least 1 positive reaction among treatment groups are provided in Table 2.

Neither the main symptoms nor the secondary symptoms were reproducible. The main symptom was fully recoverable in 11 of 39 cases (28.2%), and partially recoverable in three cases (8%). Partially recoverable referred to constellations in which two or more main symptoms (with identical numeric values on the 10-point scale) were found, but only a part of them was reproducible according to the defined criteria.

The findings were similar with respect to the secondary symptoms. They were completely reproducible in 15 of 39 cases (38.5%) and partially reproducible in one case (2.6%).

Moreover, we calculated a correlation for the two groups *open provocation* and *placebo+ histamine-containing tea* for all symptoms independent of localisation. Although the most common response among the groups was 1 (i.e. no symptom) in both cases, we were only able to determine a correlation coefficient of 0.4, which means the lack of any correlation. Due to the random reactions and thus the lack of reproducibility, primary and secondary objectives could not be evaluated.

**Table 2.** Number (*n*) and percentage (%) of subjects with at least 1 positive reaction among blinded treatment groups

|  | <i>n</i> | <i>n</i> of positive reactions | % of positive reactions |
|--|----------|--------------------------------|-------------------------|
| <i>DAO+ histamine-containing tea</i>     | 39       | 30                             | 76.9                    |
| <i>DAO+ histamine-free tea</i>           | 39       | 25                             | 64.1                    |
| <i>Placebo+ histamine-containing tea</i> | 39       | 30                             | 76.9                    |

**Table 3.** The difference in the sum of symptoms (total score) in the 3 test conditions is statistically significant (according to Friedman)

|                         |       |
|-------------------------|-------|
| <i>N</i>                | 39    |
| Chi squared             | 6.592 |
| d.f.                    | 2     |
| Asymptotic significance | 0.037 |
| Exact significance      | 0.036 |

Regarding the *total* symptom scores (tertiary objective, Table 1 and Fig. 2), the differences between the three treatment groups (*DAO+ histamine-containing tea* versus *DAO+ histamine-free tea* versus *placebo+ histamine-containing tea*) were statistically significant ( $P=0.036$ , Table 3). Paired comparisons of treatment groups according to Wilcoxon revealed the following (Fig. 2): *placebo+ histamine-containing tea* showed a statistically significant difference compared to *DAO+ histamine-free tea* towards stronger symptoms with the first test configuration ( $P=0.01$ ). The situation was similar with the paired comparison of *placebo+ histamine-containing tea* and *DAO+ histamine-containing tea*. There was a statistically significant reduction of symptoms with the configuration *DAO+ histamine-containing tea* ( $P=0.014$ ). By contrast, the symptoms of the test configuration *DAO+ histamine-free tea* were not different from the combination with *DAO+ histamine-containing tea* ( $P=0.19$ ).

## Discussion

A wide range of symptoms has been described after the intake of histamine-rich food, alcoholic beverages or histamine-releasing or DAO-blocking drugs. The symptoms are variable and heterogeneous. As a result, the diagnosis of histamine intolerance may be underdiagnosed [13].

Otherwise, identical symptoms may also occur within the scope of food allergies as well as in intolerance reactions against other biogenic amines such as tyramine [13], where the evidence for these is considered weak based on double-blind, placebo-controlled provocations (DBPCPs) [14]. Some biogenic amines are expected to be capable of triggering various symptoms by inhibiting DAO [15] or by directly liberating histamine from the mucosa [16]. However, the actual allocation of a certain symptom to a defined biogenic amine is impossible. In addition, many types of foods contain antioxidants, preservatives such as sulphites, colorants and carrier substances with the capability of triggering “pseudo allergic” reactions [17, 18]. All these factors, however, cannot explain the results of our study, as only pure peppermint tea with or without histamine was used.

Due to the heterogeneous symptoms and the in part controversial data records, additional DBPCPs using histamine are required. Due to the variable histamine content of natural food products they should be conducted in a standardised form [13].

For our study, we targeted patients with a positive history with respect to the intolerance of histamine-rich food. These preselected subjects were provoked with 75 mg of histamine, an internationally recommended regimen [13]. The development of subjective symptoms within 24 h was

defined as condition for the subsequent DBPCP with liquid histamine. Patients without symptoms were excluded. Subjects who developed subjective symptoms during this provocation underwent further double-blind testing.

The statistical evaluation of the data reveals a lack of symptom reproducibility. Neither the main nor the secondary symptoms could be reproduced reliably. Therefore, it was impossible to evaluate the changes of main and secondary symptoms defined as target criteria before the start of the study (primary and secondary objectives).

The calculation of correlations between the individual symptoms equally demonstrated a lack of any correlation. These findings are consistent with the observation that 64.1% of subjects reacted with at least one symptom to the histamine-free provocation solution. In turn, the positive provocation with a histamine-containing provocation solution and placebo capsules was no longer possible for 23%, even though all patients from this group clearly responded to at least one of the two other test configurations. The majority even responded after provocation with histamine-free tea.

Based on these observations it can be concluded that our test configuration was unable to achieve clear histamine-associated symptoms. The reason remains unclear. One may speculate that 75 mg of histamine are not enough to consistently provoke symptoms in histamine intolerant subjects or that some patients were not histamine intolerant despite their reactions after open provocation. To a certain extent, the described symptoms occurred randomly. Similar observations were made with a DBPC wine test in patients suffering from chronic urticaria as well as subjects with "wine intolerance". It was not possible to demonstrate a correlation between the wine intolerance and the histamine content of wine. Similarly to our study, 87% of these subjects equally reacted to placebo [19].

These results let us suppose that psychosomatic factors play an important role in the genesis of symptoms in a considerable proportion of patients with suspected histamine intolerance. The expectation of a reaction leads to increased self-observation and triggers subjective symptoms.

Focusing on the reproducibility of main and secondary symptoms was unsuitable to approve or exclude histamine intolerance. A similar observation was recently published after investigation of histamine sensitivity in healthy subjects [20]. Half of the study population had responded to a provocation with 75 mg liquid histamine. It was assumed that histamine intolerant patients possibly do not respond with the same effector organ, but that the histamine-sensitive biological system "human body" is affected as a whole and responds in various organ systems on different occasions. However, only very few subjects ( $n=10$ ) had been provoked.

The evaluation actually reveals a statistically significant difference between the total scores of the three test configurations (tertiary objective). It revealed that subjects provoked with histamine-containing tea experienced less symptoms with the simultaneous intake of two DAO-containing capsules instead of placebo capsules ( $P=0.014$ ). Not surprisingly, fewer symptoms were observed after

provocation with a histamine-free solution compared to the histamine-containing solution ( $P=0.01$ ). Both calculations were statistically significant. The fact, that no difference was seen between histamine-free and histamine-containing tea after supplementation of DAO ( $P=0.19$ ), point in the same direction. Regarding those for all groups constant test parameters except the test configurations *placebo capsules*, *DAO-containing capsules*, *histamine-free tea*, and *histamine-containing tea*, these changes of test configurations must have been the reason for the differences in total scores.

A limitation of the current study is the lack of a negative control group provoked with *placebo capsules* and *histamine-free tea*. The negative control in this study (DAO+ histamine-free tea) is not a real negative control, as the DAO capsules could have improved underlying basal symptoms which could be present in some patients with histamine intolerance also under basal conditions and which could be worsened by intake of histamine-rich food, alcohol or drugs releasing histamine and/ or blocking DAO. Furthermore, we did not divide into objective and subjective parameters, so that strong clinical end points are lacking. However, the importance of the psychological aspect in the field of histamine intolerance has been demonstrated using these protocols.

## Conclusions and perspective

A well-established test procedure, oral provocation with 75 mg of liquid histamine, failed to reproduce histamine-associated subjective symptoms in several of our pre-selected histamine-sensitive patients. They often reacted unexpectedly and randomly (e.g. symptoms after provocation with pure tea). A psychosomatic influence has to be suspected. Moreover one may suggest that histamine-intolerant subjects reacted with different organ systems on different occasions. As a consequence, reproducibility of single symptoms alone may not be appropriate to diagnose histamine-intolerance whereas a global symptom score could be more appropriate. The fact that the intake of DAO capsules compared to placebo led to a statistically significant reduction of total symptom scores may indirectly point in the same direction.

To clarify these points additional double-blind and placebo-controlled provocation studies are required, where the subjects should be exposed to different amounts of histamine. Additionally, these investigations should be done in a larger collective of healthy subjects compared to the study of Wöhrle et al. [20], where half of 10 subjects had reacted to 75 mg histamine. A recent publication suggests DBPCP with oral histamine and determination of plasma histamine concentration and of objective physical parameters such as heart rate, blood pressure, and erythema [21]. Patients will benefit from a clear, evidence-based diagnostic and therapeutic procedure [22].

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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